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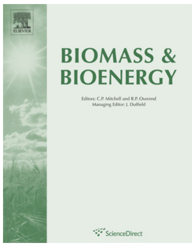

Biological degradation of torrefied wood and charcoal

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ABSTRACT

Torrefaction is an interesting option to pretreat biomass in order to obtain a fuel with improved handling and combustion characteristics, but there are a number of questions related to the supply chain and especially storage. To investigate the susceptibility of torrefied and charred spruce and birch to biological degradation, two experiments were conducted: a controlled laboratory fungal growth experiment with four different wood utilizing fungi and a preliminary field experiment with uncontrolled conditions. In laboratory, changes in moisture content, carbon and nitrogen contents and mass were measured, and the growth of the fungi was determined visually. Increasing pyrolysis temperature decreased fungal growth, but loss of carbon was noted in all of the samples. Fungal growth increased the moisture contents of samples. In the field experiment dry matter loss and increase of moisture content was noted. Torrefied wood and charcoal seem to not have full resistance towards fungal degradation. This creates additional problems that have to be taken into account when planning the supply chain of the material, as outside storage may not be advisable.

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1. Introduction

Pre-treating biomass by torrefaction has provoked much interest in recent years, because the resulting material has improved properties compared to those of the feedstock. In the process, biomass is roasted in a relatively mild temperature range of 200–300 °C, in absence of oxygen and at atmospheric pressure [1,2]. The end product has coal-like characteristics and thus could be efficiently co-combusted in existing coal-fired power plants [2].

An important part of the supply chain of any fuel is the storage. This is also a very challenging part when biomasses are in question, as large quantities are needed for continuous operation. Biomasses have a tendency to absorb moisture and degrade as a result of biological activity. Still, outdoor storage in large piles is a preferred option for e.g. wood chips due to the low costs of this method. Compared to coal, that can be stored outside in large uncovered piles with ease, degradation of untreated wood chips starts quickly, and loss of dry matter, deterioration of quality, and sometimes even spontaneous ignition take place [3,4]. Storing woody biomasses in silos or covered piles increases costs and thus is not a preferred method for large power plants.

Torrefaction changes the properties of biomass from hydrophilic to hydrophobic due to which easy storage has

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been one of the acclaimed benefits of this method [5,6]. Hydrophobic traits appear in the dehydration reactions where the destruction of many hydroxyl groups and formation of unsaturated, non-polar structures take place [2]. As decaying agents require minimum 15–20% moisture content (MC; wet basis, W.B.) [7–9], torrefied wood should be inert to biodegradation. In addition, the heat-induced caramelization process degrades low-molecular carbohydrates, which lose water and become more aromatic [10,11]. Heat-treated wood should therefore also be less suitable for mold fungi that mainly use easily assimilable substances, such as simple sugars, starch, fats, and proteins as a source of nutrition [12]. However, there are several bacteria and fungi that can also modify and decompose dry and seemingly unfavorable materials such as chemically modified wooden structures, lignite, and even hard coal [12–14]. Several micro-organisms have also adapted into living on charred wood from forest fires [12,15] and are potential utilizers of torrefied wood and charcoal in storage. When storing untreated woody material, many white- and brown-rotters can cause severe mass losses [16]. Although molds usually do not significantly affect the properties of wood, they cause health problems for workers and pose a threat for working safety [17].

The objective of this work was to study the ability of different fungi to degrade and utilize pyrolyzed wood (i.e. torrefied wood and charcoal). Based on this, the aim was to draw conclusions on the material's suitability for outdoor storage and on the durability against decay in respect to four different treatment temperatures. Two experiments were carried out: a controlled laboratory fungal growth experiment with four different wood utilizing fungi and favorable conditions, and an uncontrolled field experiment. Dry matter loss and changes in carbon, nitrogen and moisture contents were recorded.

2. Material and methods

2.1. *Sample pre-treatment with pyrolysis*

One mature spruce tree (*Picea abies* Karst.) and several young birches (*Betula pubescens* Ehrh.) were manually felled in Helsinki, Finland (approx. 60°21′6 N; 25°02′4 E). The stems and thick branches (ø min. 5 cm) were manually cut into blocks of approximately 5 × 5 × 5 cm and dried to an average MC of 6% (dry basis, D.B.) in a cold-air dryer. The size was mandated by the pyrolysis apparatus, an indirectly heated pilot scale reactor managed by Kouvola Region Vocational College's bioenergy project Biosampo. The material was pyrolyzed in 25 kg batches, at 220 °C, 260 °C and 300 °C (torrefaction) and 450 °C (charcoal). The pyrolysis was executed in steps: 110 °C 60 min, 170 °C 60 min and peak temperature with a holding time of 3 h. Long steps were necessary to ensure complete evaporation of water and thorough heat conduction within the blocks. After 3 h heating was switched off and the reactor was left to cool. Treatment at 450 °C required an extra step at 290 °C in order to prevent damage to the reactor. The effect of the long residence time on the extensive charring of the more reactive birch was tested with an additional comparative test run at 300 °C, with half the residence time (30 × 30 × 90 min).

Sample blocks were taken randomly from each temperature batch and split with a knife into smaller pieces. Also 100–200 μg samples of each block were ground for carbon–nitrogen (C/N) analysis performed with VarioMAX (Elementar Analysensysteme GmbH, Germany).

2.2. *Laboratory fungal growth experiment*

In the laboratory experiment samples split from the original blocks, measuring approximately 1 × 2 cm, were inoculated with four selected fungal species and incubated in an environment excluding outside disturbance factors, such as variation in temperature and humidity. The focus in selection was on fungi that would utilize charred and heat-treated wood and could be found in Finnish environment. The original strains were: *Phanerochaete chrysosporium* Burds. F1767 (FBCC283), *Pycnoporus cinnabarinus* (Jaqc.) P. Karst 331 (FBCC130), *Gloeophyllum sepiarium* (Wulfen) P. Karst PO121 (FBCC 190) and the genus *Trichoderma* spp. (Pers.) (FBCC1529). The strains were obtained from Fungal Biotechnology Culture Collection (FBCC) maintained in the Department of Food and Environmental Sciences, Division of Microbiology, University of Helsinki. *P. chrysosporium* and *P. cinnabarinus* are white rotters, *G. sepiarium* a brown rotter and *Trichoderma* spp. mold fungi. The fungi were grown on Petri dishes with 2% (wt/vol) malt extract agar medium for four weeks (80% RH, 25 °C). New plates with 2% water agar medium were prepared and small plugs of each fungus were inoculated on the dishes. The growth medium, the sample pieces and untreated reference pieces were autoclaved (120 °C, 20 min). Four parallel groups were established and marked I–IV. The moisture contents of the wood pieces (3–4 pieces per plate) were determined prior to the experiment by drying (105 °C, 24 h) and using the equation $MC(W.B.) = (M1 - M0)/M1 * 100$, where $M1$ is the wet mass and $M0$ the oven dry mass. The dishes were sealed with Parafilm M (SPI supplies, USA). The dishes were placed in a climate chamber (WEISS WK11 340, Weiss Klimatechnik GmbH, Germany) at a relative humidity (RH) of 80% and temperature of 25 °C. The growth of the fungi was evaluated by photographing the dishes after 0, 30 and 60 days of incubation. Photographing and measuring the MC required opening the dishes and they were disposed after measurements. Mass loss was measured by weighing the dishes before and after the experiment.

After 30 days (group IV) and after 60 days (groups I, II and III) were weighed and the MC of the sample pieces was determined. The plates were discarded after measurement due to contamination. A 5-step matrix for visual assessment was used to evaluate the advance of fungal growth: 1 – very sparse growth; 2 – sparse growth; 3 – mediate growth; 4 – considerable growth; 5 – abundant growth with discoloration of the medium. If there was no visible growth on the wood samples, but some mycelium on the water–agar medium, the evaluation was 1 in the matrix (very sparse growth), although this growth was most likely sustained by the medium instead of the substrate.

Approximately 1 × 1 × 1 mm pieces were cut from the inner surface of eight random spruce and birch samples with signs of fungal growth after 30 (4 samples) and 60 days (4 samples) of incubation. These pieces were inspected with field emission scanning electron microscopy (FE-SEM) to see whether the hyphae of the fungi had penetrated beneath the surface of the pieces. The pieces were coated with a 5 nm platinum–palladium or gold–palladium coating (Cressington sputter coater 208 HR, UK) prior to imaging with Hitachi S-4800 FE-SEM (Hitachi, Japan).

Sample pieces of every pyrolysis–fungus-combination, with visible fungal mass scraped off, were ground for C/N-analysis to determine the change in carbon and nitrogen contents (in percentages of mass). As carbon compounds are of vital importance to fungi as a source of energy, the content was expected to decrease. No expectations were made concerning nitrogen, only the direction of the change was under scrutiny. Results from visual assessment, MC measurement and C/N-analysis after 60 days were averaged for the three repetitions. The relevance of the changes in carbon and nitrogen contents and the change in mass in all temperature-fungus combinations from 0 to 30 and from 0 to 60 days was analyzed with one-sample and paired samples *t*-tests. The hypothesis was that no change would occur.

2.3. *Field experiment*

In addition to the laboratory fungal growth experiment, a preliminary field experiment was implemented, with the intention of gaining data for subsequent research. Mesh bags (mesh size 0.5 × 1 cm), each with about 20 L of wood blocks (untreated, 220, 260, 300, 450 °C), were placed outside on a compact sand/gravel ground for natural weathering. The aim was to determine the dry matter loss and changes in MC after four months of outside storage, during which time the bags were left untouched, from the beginning of July to beginning of November. The average temperature and rainfall during this time is presented in Fig. 1. After four months the bags were taken indoors, weighed, and dried at 105 °C to constant mass to determine oven dry mass loss and MC.

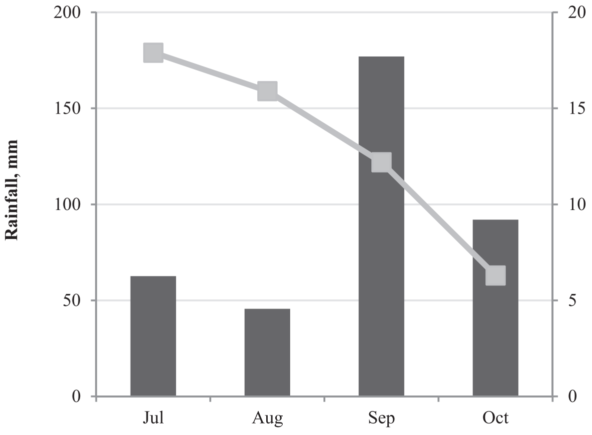


Fig. 1 – Average rainfall (mm, columns) and temperature (°C, line) in July–October in Helsinki, Kumpula weather station (data from Ref. [18]).

3. Results

3.1. *Pyrolysis pre-treatment*

The dry matter loss after the pyrolysis at 220–450 °C ranged from 46 to 73% (spruce) and from 54 to 74% (birch). The carbon content increased with increasing temperature, from 48 to 91% (spruce) and 54–88% (birch). The nitrogen content decreased with increasing treatment temperatures for spruce (from 0.4 to 0.1%) and increased for birch (from 0.4 to 0.8%). The carbon content in the comparative run was 2.9% smaller, nitrogen content 50% larger and dry matter loss 4% smaller compared to the original long run (birch, 300 °C, 60 × 60 × 90 min).

3.2. *Laboratory fungal growth experiment*

The growth of the fungi was evaluated by visual assessment, measuring the carbon, nitrogen and moisture contents, calculating the dry matter loss and through FE-SEM images. In the visual assessment *P. cinnabarinus* and *G. sepiarium* were found growing on both tree species. *P. cinnabarinus* seemed to prefer birch and *G. sepiarium* spruce, as they also do in natural conditions. *Trichoderma* spp. and *P. chrysosoporium* did not visually seem to prefer either of the tree species. Visual evaluation showed that on the wood samples treated at 450 °C the growth of all fungi was very poor and mainly determined as 1 in the matrix (very sparse growth, i.e. growth sustained by medium). Visible hyphae were still detected on the samples (2 – sparse growth) with *Trichoderma* spp. and *P. chrysosporium*. The other material treated at 220–300 °C had fungal growth ranging from 1 to 3 (mediate growth), depending on the fungal species. The untreated samples gave out results of 4–5 (considerable growth to abundant growth) with all fungi.

The C/N-analysis indicated decreased nitrogen content in most samples. The decrease was statistically significant from 0 to 30 days and from 0 to 60 days ($p \leq 0.05$) on all fungus-temperature combinations, but for birch (0–30 days). The largest decreases in the carbon content after 60 days of incubation were recorded from the untreated samples (birch), and samples treated at 220 °C (birch) and 260 °C (spruce). The decrease was significant ($p \leq 0.05$) on all other samples except birch samples after 30 days.

The average moisture content of the sample pieces after autoclaving and before inoculation was 9.0% (untreated) and 2.1–3.4% (pyrolyzed). After two months of incubation the MC of the untreated samples ranged from 45% to 72%, and of the treated samples from 16% to 66% (Fig. 2). Reference samples without inoculated fungi had MC of 9.8–37%. Decrease in mass ranged from 0.3 to 2.9% after 30 days and 1.3 to 2.9 (standard deviation ≤ 1.4) after 60. The decrease was statistically significant ($p \leq 0.05$) on all samples.

In FE-SEM, fungal hyphae were found in 50% of the samples. FE-SEM pictures from four selected samples with detectable growth are presented in Fig. 3.

3.3. *Field experiment*

The mesh bags left outside for four months gained mass 6.8–200% due to uptake of moisture. The period during which

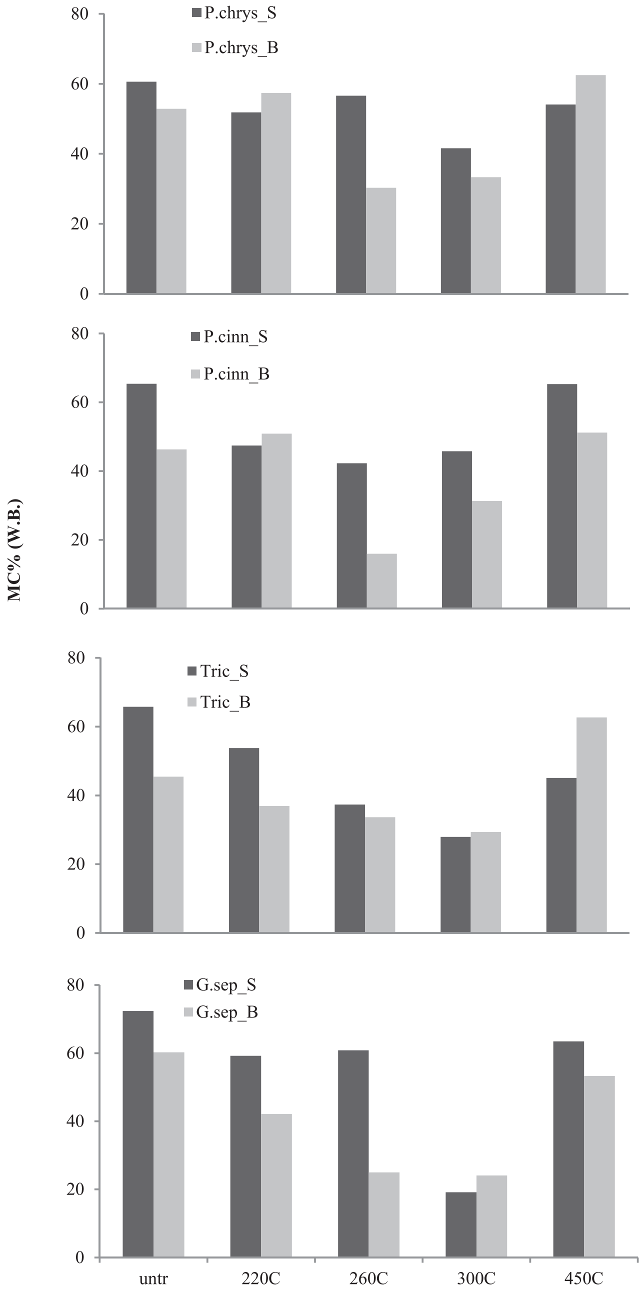


Fig. 2 – Moisture content (MC) of pyrolysed spruce (S) and birch (B) samples with selected fungi after 60 days of incubation in 80% RH. (untr = untreated reference, P.chr = *P. chrysosporium*, P.cin = *P. cinnabarinus*, Tric = *Trichoderma* sp, G.sep = *G. sepiarium*).

the bags were outside was very rainy and the blocks were practically soaked when taken back indoors. Fungal mycelia could clearly be seen in the untreated pieces as well as in the pieces pyrolyzed in 220 °C but the species were not identified. The MC of the samples ranged from 16 to 69% (Fig. 4). Compared to the untreated control samples, except for spruce samples treated at 450 °C and birch samples treated at 300 °C, the MC was lower in the torrefied samples. The dry matter loss after outdoor storage, compared to the original dry mass, was 0–22%. The loss of dry matter increased with increasing treatment temperature (220–300 °C) but again decreased with samples treated at 450 °C.

4. Discussion

4.1. *Pyrolysis pre-treatment*

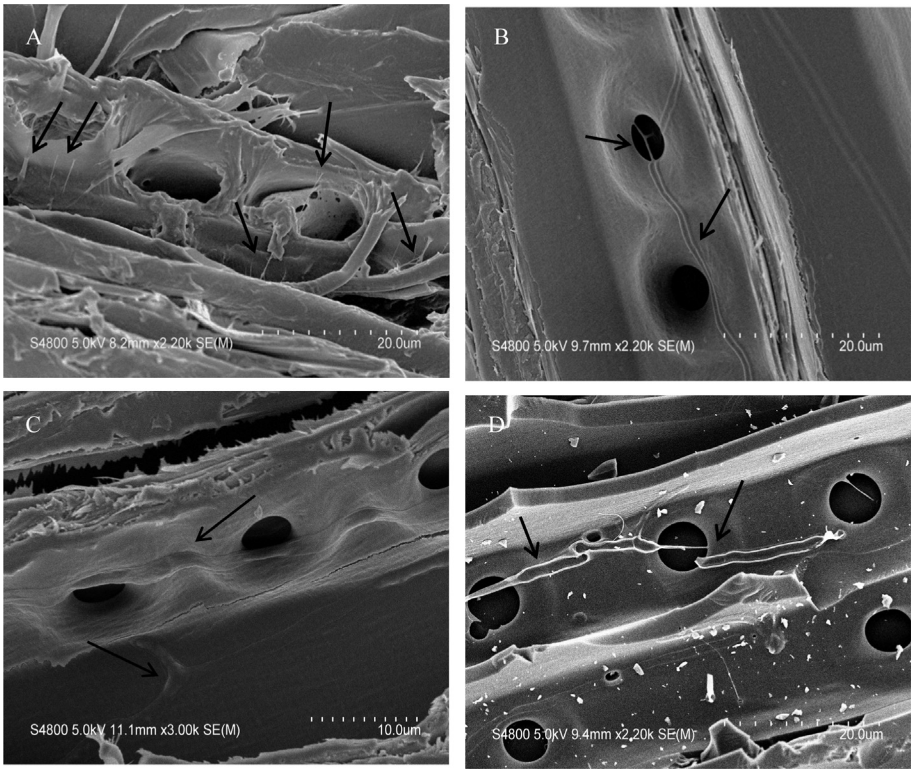
The results of the pyrolysis experiment conducted on spruce and birch wood did not differentiate from the previous studies [2,19,21]: the effect of torrefaction was more pronounced on birch, while spruce wood charred more slowly. The main differences between the two tree species can be attributed to differences in the structure of hemicelluloses. Hardwoods have more xylan which is more reactive than softwood glucomannan. As xylan starts decomposing rapidly at lower temperatures hardwoods generally lose more mass in pyrolysis [19]. Some bias was related to the heterogeneous pyrolysis result of spruce at 260 °C, as some pieces were thoroughly charred while others were not.

The pyrolysis had some effect on the nitrogen content. Longer reaction times promote the release of nitrogen into the volatile fraction (50% more remained after the shorter comparative run), but a part is still retained in the solid, depending on the manufacturing conditions [20]. The effect of holding time on carbon content was quite small. The comparative drive at 300 °C produced a carbon yield 2.9% smaller than that of the original run. Longer residence time reduces the yield of solids and increases the yield of volatiles [21], but several authors have concluded that holding time is of minor concern compared to peak temperatures [e.g. [22,23]].

4.2. *Dry matter loss*

The loss of dry matter in storage has significant economic consequences and when considering the added cost of pretreatment, the biological degradation of torrefied wood in storage could be detrimental to the profitability of the supply chain. The dry matter loss that occurred during the fungal growth experiment in the climate chamber was small but statistically significant. Condensation of water in the dishes, and absorption into the samples, and the mass of fungal mycelia distort the results to some extent, leaving the absolute change uncertain. Due to destructive methods the same samples could not be weighed twice, and comparison between 30 and 60 days was impossible. Relatively long periods of time are usually required for mass loss to show even if the wood's structure has otherwise already altered [24]. Longer exposure times and more careful exclusion of error sources would give more detailed answers.

The dry matter loss of the samples left outside clearly differed from the laboratory experiment, as the losses were quite high. Rainy summers and autumns provide favorable conditions for fungal growth and the duration of the experiment (4 months) was long enough for microbial activity to cause noticeable changes in dry matter. The losses were more pronounced with birch, which might in some part be connected to the increased nitrogen content as nitrogen was reduced in the spruce samples. Nitrogen is a very important nutrient and its low concentration can limit fungal growth [12].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C
S4800 5.0kV 11.1mm x3.00k SE(M)
10.0um
D
S4800 5.0kV 9.4mm x2.20k SE(M)
20.0um

Fig. 3 – FE-SEM of A) *G. sepiarium*, untreated birch, 30 days; B) *P. chrysosporium*, spruce, 260 °C, 30 days; C) *Trichoderma* spp., birch, 220 °C, 60 days; D) *G. sepiarium*, spruce, 450 °C, 60 days. The hyphae are indicated with black arrows.

It could be possible that as the pyrolysis breaks the hemicellulose and cellulose into smaller compounds, microbes could be able to utilize these substances more easily. After 300 °C, however, the thermal decomposition would become more extensive and less dry matter would be lost from the wood material through microbial degradation (Fig 4). Caramelization and aromatization of the compounds might also have an inhibiting role after this temperature if the naturally occurring fungi are unable to utilize thermally modified compounds. For example in a study by Singh et al. [25], wood samples treated at 300 °C suffered no mass loss when subjected to a brown- and a white rotter, although compositional analysis showed that 43% of total sugars were still held in the material. The choice of fungi was an important factor in this study as e.g. the ones associated with forest fires are clearly adapted in using components that are modified or otherwise more difficult to extract.

During storage the losses are mainly caused by microbial breakdown and chemical oxidation. According to Thörnqvist [26], in large wood chip piles the losses of dry matter can be as high as 24% during April–October (~3% per month) when stored for 6–9 months with 45–55% moisture content. Similar results were obtained by Filbakk et al. [27], where up to 3% of dry mass of residues was lost per month during autumn. A small part of the dry matter lost in the field experiment may be ascribed to leaching, the magnitude of which was not determined here.

4.3. *Moisture content*

The moisture contents of the inoculated samples were high, up to 66%, after treatment in the climate chamber, although torrefied biomass has been reported to recover only small amounts of moisture [1,2]. It is important to distinguish between the adsorption of water molecules to the cell walls and the absorption of liquid water in the bulk material. Here, the increase in MC can be attributed mainly to water absorbing in the capillaries as the axial elements of the sample pieces were in direct contact with the water that condensed in the dishes, and partly due to fungal activity, as wood constituents are degraded into CO_2 and metabolic water [28]. The reference samples did not show that much increase in MC (max. 37%) compared to the inoculated ones.

Industrial wood chip piles provide a point of comparison in this study, since there are yet no data from large storages of torrefied wood. In wood chip piles, the MC varies between seasons (high in autumn, low in summer) and the losses are proportional to the initial MC of the chips [26]. In the very small piles studied here, the highest MC in birch wood treated at 300 °C and spruce at 450 °C can be ascribed to high pyrolysis temperatures that volatilize the cell walls, enlarging the pores that can absorb larger amounts of water, and also to the mass based equation. The more rapid degradation of birch hemicelluloses can be seen in the low MC at the lowest treatment temperatures, as the porosity is not yet prominent but adsorption in the cell wall is hindered by destruction of hydroxyl groups. After 260 °C, the volatilization of cell walls becomes more extensive, creating more available space for free water at 300 °C. At 450 °C, however, the pores might have become large enough for capillary forces to weaken, explaining the sudden decline on the more reactive birch. According to Fig 4, increasing porosity

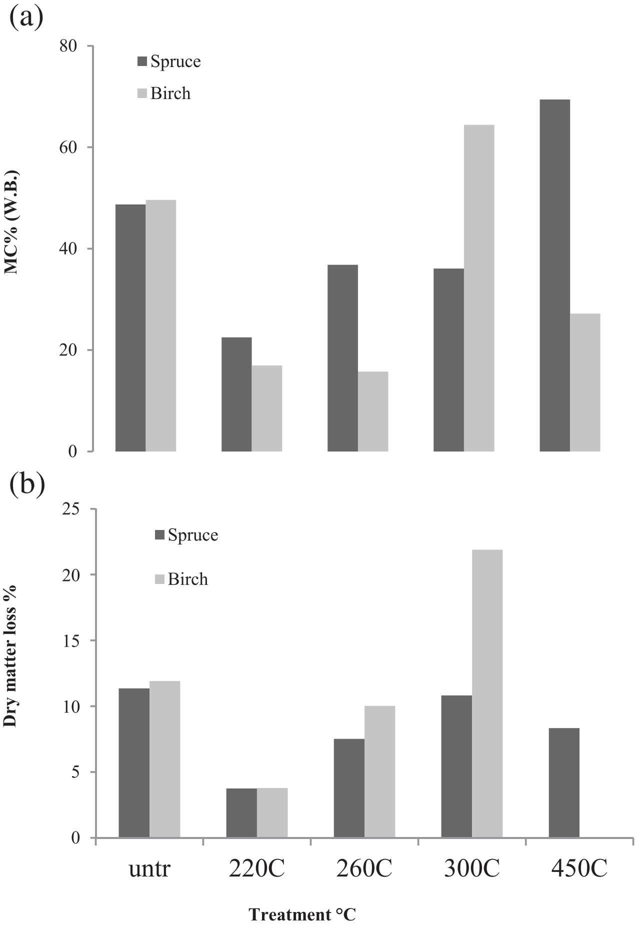

Fig. 4 – Moisture content (a) dry matter loss (b) of samples after four months of outside storage.

seems to have a great impact on the dry matter loss in the torrefaction range.

4.4. *Fungal growth*

Three kinds of fungi were tested here, mold, white-rot and brown-rot fungi. Wood rotting fungi are divided into three groups (white, brown and soft rot) according to their ability to utilize different components of wood and produce characteristic types of decay. Brown-rot fungi are predominantly associated with softwoods and white-rot fungi with hardwoods, while some fungi have no host preference between wood types, such as *P. chrysosporium* [29]. The practical differences between them, from the point of view of storing pyrolyzedpyrolyzed wood outdoors, are their growth rates and the caused material losses. In the case of molds the most important aspect is their negative health impact. Workers exposed to and inhaling spores of fungi along with wood dust experience diseases such as organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis [17].

According to this study, the most adaptable fungus is *Trichoderma* spp., which could be found also on all of the charcoal samples. Some species of the genus *Trichoderma* spp. are among the most common mold fungi found in wood chip piles [3,7]. Molds use primary metabolites, such as soluble sugars and lipids as substrate. *Trichoderma* can also use aromatic compounds, methanol and formate [30], all products of pyrolysis [1,31] and although low molecular mass compounds mostly escape in the process, a part probably remains in the solid phase. The easily assimilable substances are often thought to be degraded at least in charred wood. According to Sehlstedt-Persson et al. [10], the number of monosaccharides in hemicellulose starts decreasing already at 115 °C, though limited volatilization continues until much higher temperatures [32]. However, according to Boon et al. [33] and Pastorova et al. [34], cellulosic oligosaccharides, anhydrooligosaccharides and glucose were present after heating in 250 °C, 270 °C and 310 °C, respectively, and could still be available for some fungi. Cellulose also consists of carbohydrates, but utilizing it requires extracellular enzymes such as cellulases which some *Trichoderma* species (*T. reesei*) are known to secrete [35]. It is also possible that some species of *Trichoderma* can degrade lignin [36]. The success of *Trichoderma* spp. leads to the conclusion that molds can grow on torrefied wood and charcoal, and though large losses of material are usually not found, they do pose a threat for working safety.

Loss of carbon was found in all samples with white rotter *P. chrysosporium*. Usually the optimum temperature for wood-decaying basidiomycetes is between 20 and 30 °C, but *P. chrysosporium* is thermophilic and has a growth optimum at 40 °C [3,12]; incubation at a steady 25 °C might have hindered its growth to some extent but the heat generation in large piles could in turn favor its appearance. Another white rotter, *P. cinnabarinus*, a rapid degrader of lignin, caused loss of carbon on both spruce and birch. Its natural occurrence in forest fire areas [37] makes it a viable degrader of stored torrefied wood as well as charcoal. Brown rotter *G. sepiarium* prefers coniferous wood, but caused carbon loss in both tree species. It is also found on forest fire areas [37]. It uses endoglucanases to degrade cellulose and hemicelluloses, but has also been reported to produce cellobiohydrolases that erode crystalline cellulose [38,39] and could therefore also utilize quite severely charred wood.

The laboratory experiment aimed to determine if the selected fungi can utilize pyrolyzed wood. The visual assessment, FE-SEM pictures and carbon, nitrogen and dry matter loss indicate that these fungi are able to live on torrefied and charred wood, although in most cases the amount of fungal hyphae was moderate and the growth rate was slow. The stable conditions in laboratory experiments are quite different compared to conditions in large chip piles stored outdoors. The temperature and humidity were close to optimal for most fungi and moisture content high enough to promote the growth. In a large outdoor pile the moisture content depends on the weather and varies greatly during the year. Fungal growth seems to be possible at least in favorable conditions, since carbon and nitrogen loss were recorded. The question, whether this growth is enough to cause significant material losses, requires further investigation. The outdoor experiment performed here gives some indication on the biological degradation occurring in field conditions, as more pronounced dry matter losses were recorded. However, the samples were blocks instead of commonly used chips and the sample size was small. These results should therefore be considered only as a first look into the matter of storage. Random variation cannot be entirely excluded from outdoors experiments, but from the laboratory experiment it was clear that fungi are able to settle on seemingly unfavorable material, that is said to be hydrophobic and suitable for outside storage. Each differently treated sample had advantages and disadvantages: the more mildly treated samples experienced more carbon loss, but took in less moisture in outside storage, contrary to the more severely treated samples. The uneven pyrolysis of spruce at 260 °C slightly distorts the results, but birch treated at the same temperature shows quite good properties in respect to moderate sorption and carbon loss. More studies are needed to assess the growth of fungi on torrefied wood and charcoal from different treatment temperatures, also in industrial sized piles, where the heat formation can create a versatile environment for the fungal community. Also the extent of leaching in storage piles should be assessed to determine its effect on the total dry matter loss of torrefied wood and charcoal.

5. Conclusions

The laboratory and field experiments show that in storage, pyrolyzed wood (i.e. torrefied wood and charcoal) are subjected to biological degradation and dry matter losses. The laboratory experiment evaluated the ability of four selected fungi to utilize torrefied wood and charcoal samples in laboratory conditions. As a result it can be stated that in favorable conditions, these fungi can utilize the carbon and nitrogen components of torrefied wood and charcoal. The recorded carbon loss decreased towards the more severe end of the pyrolysis treatments. Although torrefaction increases the hydrophobic nature of wood, abundant humidity, capillary absorption and presence of various fungi, in turn, moistens the wood, which accelerates dry matter loss. Loss of dry matter in the laboratory experiment was quite small, whereas a loss of up to 22% in the field experiment samples clearly indicated a negative impact on the supply chain. The loss of dry matter and carbon as well as the increasing moisture content decrease the calorific value of the fuel and thus increase associated costs. More studies are required to quantify the biological deterioration.

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